

NT-3 Immunohistochemistry with Amplification

Day 1

- 1) Remove slides from -80°C freezer and allow to warm to room temperature (~10 minutes)
- 2) Bake slides overnight at 42°C

Day 2

- 3) Wash slides 2 x 10 minutes in PBS
- 4) Treat with 0.3% H₂O₂ for 10 minutes
- 5) Wash slides 2 x 10 minutes in PBS
- 6) Permeabilize tissue for 30 minutes in 0.3% PBST
- 7) Block slides with TNB Buffer (spin) for 30 minutes at room temperature
- 8) Block slides with α NT-3 (1:1000 in TNB buffer **spin down before using) over the weekend at 4°C

Day 3

- 9) Wash slides in 0.3% PBST 3 x 5 minutes
- 10) Block slides with biotinylated (rabbit) secondary (1:200 in TNB buffer) for 60 minutes at room temperature (spin)
- 11) Wash slides in 0.3% PBST 5 x 5 minutes with agitation
- 12) Block slides with SA-HRP (1:100 in TNB buffer) for 30 minutes at room temperature (spin)
- 13) Wash slides in 0.3% PBST 5 x 5 minutes with agitation
- 14) Block slides with fluorophore tyramide (1:50 in amplification diluent **spin down before using) for 10 minutes at room temp
- 15) Wash in 0.3% PBST 5 x 5 minutes with agitation
- 16) Cover slip with Mowiol